Coelomycetous Fungi in the Clinical Setting: Morphological Convergence and Cryptic Diversity

Nicomedes Valenzuela-Lopez,^{a,b} Deanna A. Sutton,^c José F. Cano-Lira,^a
Katihuska Paredes,^a Nathan Wiederhold,^c Josep Guarro,^a Alberto M. Stchigel^a

Unitat de Micologia, Facultat de Medicina i Ciències de la Salut and IISPV, Universitat Rovira i Virgili, Reus, Spain^a; Microbiology Unit, Medical Technology Department, Faculty of Health Science, University of Antofagasta, Antofagasta, Chile^b; Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA^c

ABSTRACT Human infections by coelomycetous fungi are becoming more frequent and range from superficial to systemic dissemination. Traumatic implantation of contaminated plant material is the most common cause. The typical morphological feature of these fungi is the production of asexual spores (conidia) within fruiting bodies called conidiomata. This study aimed to determine the distribution of the coelomycetes in clinical samples by a phenotypic and molecular study of a large set of isolates received from a U.S. reference mycological institution and by obtaining the *in vitro* antifungal susceptibility pattern of nine antifungals against a selected group of isolates. A total of 230 isolates were identified by sequencing the D1 and D2 domains of the large subunit (LSU) nuclear ribosomal RNA (nrRNA) gene and by morphological characterization. Eleven orders of the phylum *Ascomycota* were identified: *Pleosporales* (the largest group; 66.1%), *Botryosphaeriales* (19.57%), *Glomerellales* (4.35%), *Diaporthales* (3.48%), *Xylariales* (2.17%), *Hysteriales* and *Valsariales* (0.87%), and *Capnodiales*, *Helotiales*, *Hypocreales* and *Magnaporthales* (0.43% each). The most prevalent species were *Neoscytalidium dimidiatum*, *Paraconiothyrium* spp., *Phoma herbarum*, *Didymella heteroderae*, and *Epicoccum sorghinum*. The most common anatomical site of isolation was superficial tissue (66.5%), followed by the respiratory tract (17.4%). Most of the isolates tested were susceptible to the majority of antifungals, and only flucytosine showed poor antifungal activity.

KEYWORDS *Colletotrichum*, coelomycetous fungi, coelomycetes, mycosis, *Neoscytalidium*, *Phoma*, *Pyrenochaeta*, antifungal susceptibility

The coelomycetous fungi constitute a large number of taxa characterized by the production of conidia (asexual propagules) within a cavity lined by fungal or host tissue, called conidiomata (1), and although the majority of the human opportunistic infections are caused by fungi producing conidia on conidiophores (modified hyphae, with one or more conidiogenous cells, which develop free on the substrate), a significant number of mycoses are produced by coelomycetous fungi (2–4). Coelomycetous fungi are mostly saprobic and parasites of terrestrial vascular plants, but they can also infect vertebrates and other fungi. They are ubiquitous in soil, in salty and freshwater environments, and in sewage (4). Although the term *Coelomycetes* is still occasionally used to refer to these fungi, this name is obsolete and is currently considered to refer to an artificial fungal class. The class *Coelomycetes* is defined in terms of the morphological characterization of the asexual reproductive structures and considers the type and the shape of their conidiomata and the ontogeny of their conidia as the most useful characteristics (5, 6); the class has traditionally been divided into the orders *Melanconiales* and *Sphaeropsidales*, depending upon the production of either acervular

Received 2 November 2016 Returned for modification 21 November 2016 Accepted 29 November 2016

Accepted manuscript posted online 7 December 2016

Citation Valenzuela-Lopez N, Sutton DA, Cano-Lira JF, Paredes K, Wiederhold N, Guarro J, Stchigel AM. 2017. Coelomycetous fungi in the clinical setting: morphological convergence and cryptic diversity. *J Clin Microbiol* 55:552–567. <https://doi.org/10.1128/JCM.02221-16>.

Editor David W. Warnock, University of Manchester

Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to José F. Cano-Lira, jose.cano@urv.cat.

FIG 1 Distribution, by orders, of coelomycetous fungus isolates from clinical samples.

(cup-shaped) and pycnidial (globose to pyriform) conidiomata, respectively, and the *Pycnothyriales*, characterized by the production of pycnothyrial (shield-shaped, flattened, or hemispherical) conidiomata (5, 6). However, molecular studies have demonstrated that the taxonomy of the *Coelomycetes*, represented by nearly 1,000 genera and 7,000 species (1), is artificial. Recent studies, have distributed the coelomycetes into at least three classes of the phylum *Ascomycota*, i.e., *Dothideomycetes*, *Leotiomycetes*, and *Sordariomycetes* (7–9).

Infections by coelomycetous fungi are mostly acquired by traumatic implantation of plant/woody material or soil particles contaminated by their conidia rather than by inhalation of air-dispersed propagules (2, 4). The coelomycetes are responsible for a large variety of clinical entities, such as dermatitis, onychomycosis, keratitis, endophthalmitis, subcutaneous phaeohyphomycosis, cysts, mycetoma, sinusitis, osteomyelitis, bursitis, brain abscesses, and disseminated infections (4). The appropriate treatment of the infections produced by these fungi is unknown, mainly due to the wide spectrum of taxa involved and to the difficulties in their identification when the typical reproductive structures are not produced. However, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the European Conference of Medical Mycology (ECMM) have provided joint clinical guidelines for the management of phaeohyphomycosis, with some recommendations for the treatment of infections due to the most usual genera of coelomycetes, such as *Neoscytalidium*, *Phoma*, and *Pyrrenochaeta*, mainly based on the use of amphotericin B and triazoles (10).

For the reasons mentioned above, the spectrum of species of these fungi in the clinical setting is practically unknown (4, 11). Therefore, the objective of this study has been to determine the distribution pattern of the coelomycetous fungi isolated from clinical specimens from the United States using molecular identification of a large set of isolates based on the sequencing of the D1 and D2 (D1-D2) domains of the large subunit (LSU) of the nuclear ribosomal RNA (nrRNA) gene. In addition, we have characterized those isolates morphologically and determined the antifungal susceptibility of a representative number of them to nine antifungal drugs.

RESULTS

A total of 86 (38%) isolates of the 230 studied were able to produce pycnidial conidiomata; 10 (4%) developed acervuli, and 35 (15%) produced the typical anamorphs of *Neoscytalidium*. The other 99 isolates (43%) remained sterile. The most common species was *Neoscytalidium dimidiatum*, representing 15% (35/230) of the isolates, followed by *Paraconiothyrium cyclothyrioides* with 7% (16/230), and both were isolated mostly from superficial tissues. The third most common taxon recovered was

FIG 2 Maximum-likelihood tree obtained from the D1-D2 of LSU (555 bp) sequences of the 322 strains, where 92 strains are type or reference strains. In the tree, the branch lengths are proportional to phylogenetic distance. Bayesian posterior probability scores of ≥ 0.95 and bootstrap support values of ≥ 70 are indicated on the nodes. The GenBank accession numbers are given in parentheses. *Saccharomyces castellii* and *S. cerevisiae* were used to root the tree. The type (indicated by a superscript T) and reference strains are shown in bold type.

FIG 2 (Continued)

Phoma herbarum (6.5%, 15/230) from superficial and respiratory tract specimens, followed by *Didymella heteroderae* (5%, 12/230) and *Epicoccum sorghinum* (4%, 10/230), which were isolated from superficial tissues.

In the D1-D2 phylogenetic analysis, the isolates were distributed into 11 orders (Fig. 1), most of which belonged to the Pleosporales (66.1%) and the Botryosphaeriales

FIG 2 (Continued)

FIG 2 (Continued)

(19.57%), followed by the *Glomerellales* (4.35%), *Diaporthales* (3.48%), *Xylariales* (2.17%), and *Hysteriales* and *Valsariales* (0.87% each). The orders *Capnoidiales*, *Helotiales*, *Hypocreales*, and *Magnaporthales* were represented by only one isolate each (0.43%), and the other isolates (0.87%) were *incertae sedis* (of uncertain taxonomic position).

Figure 2 shows the phylogenetic tree inferred from the analysis of 322 D1-D2 sequences corresponding to our set of isolates and numerous selected type or reference strains phylogenetically related to them. As mentioned above, the *Pleosporales* contained the largest number of isolates ($n = 152$), which were distributed into 22 clades and belonged, probably, to 61 species of 44 different genera. These clades have been named according to the first taxon historically described.

Within the *Pleosporales*, *Phoma* clade I (phylogenetically not supported) included 27 isolates, distributed mainly in two genera: *Leptosphaerulina*, with four isolates characterized by a phoma-like asexual morph, which clustered with a reference strain of *Leptosphaerulina australis*, and *Phoma*, with 15 isolates placed close to a reference strain of *Phoma herbarum* and morphologically characterized by producing pycnidia and hyaline aseptate conidia. The taxonomic position of the other eight isolates of this clade was unresolved; in fact, they formed a separate, unsupported sister clade and displayed a phoma-like asexual morph. The University of Texas Health Science Center (UTHSC) isolate DI16-270 also showed the typical morphology of *Phoma* (*Phoma* clade II) but has been placed phylogenetically distant from the mentioned genera and probably belongs to a new genus.

The *Didymella* clade included 22 isolates, 10 of which clustered with a reference strain of *Epicoccum sorghinum*; unfortunately, the morphological features of these isolates could not be studied because they produced only sterile mycelia in all the culture media tested. Twelve isolates grouped with the type strain of *Didymella heteroderae*, producing a phoma-like asexual morph, but were particularly characterized by the production of chlamydospores in long chains.

The *Neosascochyta* clade included seven isolates, six clustering with the type strain of *Neosascochyta desmazieri* and another one placed together with a reference strain of *Ascochyta hordei* var. *hordei*. Morphologically, the species of this clade are mainly characterized by the production of one-septate conidia that vary in size.

The *Paraphoma* clade contained only one isolate, which showed an identical sequence to the type strain of *Paraphoma radicina* and morphologically was characterized by setose (covered with bristle-like structures) pycnidia and hyaline aseptate conidia.

The *Pleospora* clade was made up of five isolates and, with the exception of one of them, was distributed into three well-supported sister clades corresponding to the genera *Edenia*, *Paraphoma*, and *Trematophoma*. The isolate that clustered with the type strain of *Paraphoma fimeti* was separate from the type species of *Paraphoma* (*Paraphoma radicina*) and showed glabrous pycnidia instead the setose pycnidia produced by the rest of the species. Interestingly, instead of the ellipsoidal, subhyaline conidia typical of *Edenia* spp., the isolate UTHSC DI16-324 produced fusiform, hyaline, two- to three-septate conidia that are probably indicative of a new genus. The other isolates of this clade remained sterile.

The *Coniothyrium* clade included nine isolates, and its topology shows that the genera *Coniothyrium*, *Leptosphaeria*, and *Pyrenochaeta* are clearly polyphyletic using this conserved marker. Three of these isolates formed a well-supported sister clade together with a reference strain of *Coniothyrium telephii*, which is characterized by setose pycnidia. The other six isolates were distributed into the genera *Leptosphaeria* and *Pyrenochaeta*. These had a pyrenochaeta-like anamorph, producing conidiophores within pycnidia and hyaline aseptate conidia.

The *Phaeosphaeria* clade grouped nine isolates, with four of them clustering with *Neosetophoma* and producing confluent pycnidia and small hyaline conidia. The other five isolates were associated with the genera *Diederichomyces*, *Parastagonospora*, *Phaeosphaeria*, and *Phaeosphaeriopsis*. Only one isolate (UTHSC DI16-325), morphologically

resembling *Phaeosphaeriopsis* spp., was able to sporulate, displaying small conidiophores within pycnidia and one-septate, pigmented, variable-in-shape conidia.

The *Pyrenochaetopsis* clade included nine isolates, with four of them matching the type strain of *Pyrenochaetopsis leptospora*, another four isolates forming a supported sister clade separate from *P. leptospora*, and one not clustering to any of the type strains included in the analysis. All of the isolates displayed the typical phoma-like morphology, i.e., glabrous pycnidia and hyaline aseptate conidia, instead of setose pycnidia of the genus *Pyrenochaetopsis*.

The five isolates assigned to the *Acrocalymma* and *Medicopsis* clades were grouped with the type strains of *Acrocalymma walkeri* and *Medicopsis romeroi*, respectively, but differed in 4.5% of the nucleotide sequences of the respective strains of reference. These isolates remained sterile throughout.

The *Roussoella* clade was made up of eight isolates, two of which were associated with a supposed reference strain of *Arthopyrenia salicis* (CBS 368.94), whose correct identification was questioned by Liu et al. (12), and the remaining ones were associated with *Roussoella* spp.; only three isolates were able to sporulate and had a morphology similar to that of this genus, i.e., production of glabrous pycnidia and pigmented aseptate conidia.

Two isolates nested in the *Biatrispora* clade but remained sterile. The *Trematosphaeria* clade comprised five sterile isolates, two of which were phylogenetically related with the type strain of *Trematosphaeria pertusa* and the rest of which were associated with a reference strain of *Trematosphaeria grisea*.

The *Keissleriella* clade had only one isolate, which showed a phoma-like morphology and clustered with a reference strain of *Keissleriella cladophila*. Another isolate was associated with a reference strain of *Paraconiothyrium flavescens* and displayed a morphology similar to that of *Paraconiothyrium* (pycnidia, phialidic conidiogenous cells, and pigmented aseptate conidia); however, the taxonomic placement of that isolate remains doubtful because it grouped phylogenetically distant from the type species of the genus (*Paraconiothyrium estuarinum*). In the *Camarographium* clade, two sterile isolates were located that were related to the genera *Camarographium* and *Pseudochaetosphaeronema*.

The *Didymosphaeriaceae* clade comprised 33 isolates, of which 22 were phylogenetically related to *Paraconiothyrium* spp., 2 were related to *Montagnula* spp., and 2 were related to the type strain of *Paraphaeosphaeria neglecta*. Three isolates were distributed into each of the genera *Bimuria*, *Curreya*, and *Phaeodothis*, and four isolates formed a well-supported monophyletic sister clade separated from any known taxa of the family. Only three isolates (UTHSC DI16-261, UTHSC DI16-266, and UTHSC DI16-363) were able to sporulate, showing glabrous pycnidia and pale brown conidia displaying morphological features similar to those of *Paraconiothyrium* spp. The *Exosporium* clade comprised only two sterile isolates, one of which was related to the genus *Preussia* while the other was related to *Exosporium*. The *Anteaglonium*, *Lophiostoma*, and *Phyllosticta* clades comprised only one sterile isolate each one.

In the *Valsariales* clade, two isolates matched a reference strain of *Myrmaecium rubricosum*. These isolates were characterized by producing free, well-differentiated conidiophores instead of simply conidiogenous cells (phialides) inside the pycnidia.

The *Hysteriales* clade contained two sterile isolates, one related to an unidentified strain of *Chaetophoma* and one of uncertain taxonomical placement but phylogenetically related to *Chaetophoma*, *Gloniopsis*, and *Rhytidhysterion*.

The second largest clade, corresponding to the order *Botryosphaerales*, included 45 isolates distributed in six clades but mostly concentrated into the *Neoscytalidium* clade. The fungi included in these clades were characterized by the production of stromatic conidiomata (a hard, compact mass of cells or of vegetative hyphae), holoblastic instead of phialidic conidiogenous cells, and aseptate, hyaline to brown, thick-walled conidia. The *Botryosphaerales* included the genera *Botryosphaeria* (three isolates), *Lasiodiplodia* (two isolates), *Neofusicoccum* (one isolate), *Aplosporella* (two isolates), and *Phaeobotryosphaeria* (two isolates). Additionally, 35 isolates of *Neoscytalidium dimidia-*

tum were also placed in this order. This fungus is characterized typically by the production of holoarthric conidia (formed by disarticulation of the preexisting hyphae) in chains.

The *Capnodiales*, *Helotiales*, and *Magnaporthales* clades each included only one sterile isolate. Only the isolate of the *Helotiales* was not phylogenetically related to any previously known described species. The isolate of the *Capnodiales* was closely related to a reference strain of *Pseudocercospora oenotherae*. This genus is characterized by producing stromata in the (plant) host, subhyaline to brown conidiophores, and small or large, subhyaline to brown conidia; unfortunately, our isolate failed to sporulate. In the *Magnaporthales*, the isolate matched a reference strain of *Mycoleptodiscus indicus*. This genus is characterized by producing sporodochia (a cushion-like, densely aggregated group of conidiophores) and curved conidia; in this case, the morphological study was not possible due to the absence of sporulation of the isolate.

The *Xylariales* clade included five sterile isolates, three of which were related to the genus *Diatrype* but phylogenetically distant from a reference strain of *Diatrype disciformis*. The remaining two isolates were associated with the *Peroneutypa* clade, with one of them matching a reference strain of *Peroneutypa scoparia* and the other uncertainly placed taxonomically.

The *Diaporthales* clade grouped eight isolates, six of which belonged to the *Diaporthe* clade and were characterized by the production of hyaline conidiophores within pycnidial conidiomata, phialidic conidiogenous cells, and small conidia. The other two sterile isolates were located in the *Valsa* clade.

The *Hypocreales* clade included only a single sterile isolate that matched a reference strain of *Thyronectria austroamericana*.

The *Glomerellales* clade comprised 10 isolates, all of which belonged to the genus *Colletotrichum* and were characterized by the production of acervular conidiomata, phialidic conidiogenous cells, conidia variable in shape, and the presence of appressoria. Six of the isolates were identified as *Colletotrichum gloeosporioides*, two were identified as *Colletotrichum truncatum*, and one was identified as *Colletotrichum spaethianum*. One isolate (UTHSC DI14-247) was molecularly closely related to a reference strain of *Colletotrichum torulosum*.

Two isolates (UTHSC DI16-350 and UTHSC DI16-223) were not located in any of the previously known orders and consequently were treated as *incertae sedis*. The first one was assigned to the *Phomatospora* clade and the other, characterized by the production of sporodochia and hyaline conidia, was identified as *Phialemoniopsis curvata*.

From a total of 224 clinical isolates, 153 were recovered mainly from superficial tissues (epidermis and dermis) (66.5%), followed by 40 from the respiratory tract (17.4%), 22 from miscellaneous deep tissues or fluids (9.6%), and 9 isolates from subcutaneous tissues (3.9%) (Table 1).

Approximately half of all the fungi tested (44%; 101/230) were able to grow at 37°C (Table 1); they were distributed within the orders at the following percentages: 100% (10/10) of the *Glomerellales*, 100% (2/2) of *Hysteriales*, 100% (2/2) of the *Valsariales*, 98% (44/45) of the *Botryosphaerales*, 50% (1/2) of the isolates *incertae sedis*, and 28% (42/152) of the *Pleosporales*.

Table 2 summarizes the results of the antifungal susceptibility testing. In general, all the drugs tested, but especially terbinafine and amphotericin B, showed good activity against the coelomycetous fungi, with terbinafine being the most active (geometric mean [GM] of 0.04 µg/ml; MIC₉₀ of 0.03 µg/ml). Among the triazoles, itraconazole was the least active, with an overall GM of 1 µg/ml and a MIC₉₀ of 16 µg/ml. *Colletotrichum gloeosporioides*, *Neoscytalidium dimidiatum*, and *Didymella heteroderae* showed high MICs for all the antifungals tested. Posaconazole and voriconazole demonstrated similar *in vitro* potencies, with the only exceptions being activity against *Colletotrichum gloeosporioides* and *Neoscytalidium dimidiatum*, for which the voriconazole GMs were 2.64 and 2 µg/ml, respectively, and against *Neoscytalidium dimidiatum*, for which the posaconazole GM was 2.26 µg/ml. All the echinocandins showed good *in vitro* activity

TABLE 1 Anatomical sites of coelomycetous fungus isolates from clinical specimens

Order	Clade	No. of isolates obtained from:					37°C growth	Total no. of isolates
		Superficial tissue	Subcutaneous tissue	Deep tissue/fluids	Respiratory tract	Environment and animal		
<i>Botryosphaerales</i>	<i>Aplosporella</i>	2					+	2
	<i>Botryosphaeria</i>	3					+	3
	<i>Lasioidiplodia</i>	2					+	2
	<i>Neofusicoccum</i>	1					—	1
	<i>Neoscytalidium</i>	27		3	5		+	35
	<i>Phaeobotryosphaeria</i>	2					+	2
<i>Capnodiales</i>		1					—	1
<i>Diaporthales</i>	<i>Diaporthe</i>	3		2	1		+	6
	<i>Valsa</i>	1			1		+	2
<i>Glomerellales</i>		7	1	1	1		+	10
<i>Helotiales</i>		1					—	1
<i>Hypocreales</i>		1					+	1
<i>Hysteriales</i>			1		1		+	2
<i>incertae sedis</i>	<i>Phialemoniopsis</i>	1					+	1
	<i>Phomatospora</i>			1			—	1
<i>Magnaporthales</i>		1					+	1
<i>Pleosporales</i>	<i>Acrocalymma</i>	1					—	1
	<i>Anteaglonium</i>	1					—	1
	<i>Biatrispora</i>	1			1		—	2
	<i>Camarographium</i>	2					—	2
	<i>Coniothyrium</i>	7	1	1			—	9
	<i>Didymella</i>	17		1	4		+	22
	<i>Didymosphaeriaceae</i>	26	1	2	3	1	—	33
	<i>Exosporium</i>	1				1	—	2
	<i>flavescens</i>	1					—	1
	<i>Keissleriella</i>		1				—	1
	<i>Lophiostoma</i>		1				—	1
	<i>Medicopsis</i>	3		1			+	4
	<i>Neosascochyta</i>	6			1		—	7
	<i>Paraphoma</i>	1					—	1
	<i>Phaeosphaeria</i>	2	1	1	4	1	—	9
	<i>Phoma</i> I	13		3	10	1	—	27
	<i>Phoma</i> II	1					—	1
	<i>Phyllosticta</i>	1					+	1
	<i>Pleospora</i>	1		1	3		—	5
	<i>Pyrenochaetopsis</i>	6		1	2		—	9
	<i>Roussoella</i>	5		2	1		+	8
	<i>Trematosphaeria</i>	4		1			+	5
<i>Valsariales</i>						2	+	2
<i>Xylariales</i>	<i>Diatrype</i>		1	1	1		—	3
	<i>Peroneutypa</i>		1		1		—	2
Total no. of isolates (%)		153 (66.5)	9 (3.9)	22 (9.6)	40 (17.4)	6 (2.6)		230 (100)

against these fungi, with a GM of 0.06 $\mu\text{g/ml}$. Flucytosine was the least active antifungal tested, with elevated MICs against all isolates.

DISCUSSION

This is, to our knowledge, the largest taxonomic study on coelomycetous fungi of clinical origin. It has demonstrated, based on DNA sequencing, a wider diversity of taxa than previously reported. Although two recent reviews have reported approximately 35 species of coelomycetes involved in human infections (3, 4), the present study identifies 88 species; unfortunately, the role of many of them as pathogens for human still remains uncertain because the clinical data of the patients are not allowed to be

TABLE 2 Results of *in vitro* antifungal susceptibility testing of coelomycetous fungi

Taxon (no. of isolates)	Parameter ^a	Value for the drug ($\mu\text{g/ml}$) ^b								
		AMB	VRC	ITC	PSC	AFG	CFG	MFG	TRB	5FC
<i>Neoscochyta desmazieri</i> (5)	GM	0.44	2	0.57	0.21	0.03	0.03	0.03	0.03	1.15
	Range	0.25–1	1–4	0.25–1	0.06–0.5	0.03–0.06	≤ 0.03	≤ 0.03	≤ 0.03	0.5–2
	MIC ₉₀	0.5	2	1	0.5	0.03	0.03	0.03	0.03	2
<i>Colletotrichum gloeosporioides</i> (5)	GM	0.57	2.64	8	0.87	0.03	0.03	0.03	0.03	16
	Range	0.03–2	0.5–4	1–16	0.5–1	≤ 0.03	≤ 0.03	≤ 0.03	≤ 0.03	≥ 16
	MIC ₉₀	2	4	16	1	0.03	0.03	0.03	0.03	16
<i>Epicoccum sorghinum</i> (8)	GM	0.25	0.92	0.59	0.30	0.03	0.04	0.03	0.03	2.97
	Range	0.12–1	0.5–2	0.5–1	0.12–0.5	0.03–0.06	0.03–0.5	≤ 0.03	≤ 0.03	1–8
	MIC ₉₀	0.5	1	1	0.5	0.03	0.03	0.03	0.03	4
<i>Neoscytalidium dimidiatum</i> (16)	GM	0.22	0.59	2.56	2.26	0.13	0.2	0.47	0.08	2.83
	Range	0.06–1	0.03–16	0.06–16	0.03–16	0.03–0.5	0.03–1	0.06–8	0.03–2	0.25–16
	MIC ₉₀	0.5	4	16	16	0.25	0.5	4	0.03	8
<i>Paraconiothyrium cyclothyrioides</i> (15)	GM	0.25	0.25	0.3	0.15	0.03	0.03	0.03	0.03	2.61
	Range	0.03–8	0.06–0.5	0.06–0.5	0.03–0.5	≤ 0.03	≤ 0.03	≤ 0.03	≤ 0.03	1–16
	MIC ₉₀	0.5	0.5	0.5	0.25	0.03	0.03	0.03	0.03	4
<i>Didymella heteroderae</i> (11)	GM	1.76	1.87	3.31	1.07	0.34	0.13	0.14	0.03	4
	Range	0.5–8	0.06–16	0.5–16	0.5–2	0.03–8	0.03–4	0.03–2	≤ 0.03	1–16
	MIC ₉₀	4	16	16	2	8	4	2	0.03	16
<i>Phoma herbarum</i> (10)	GM	0.43	0.57	0.81	0.40	0.04	0.04	0.03	0.03	2
	Range	0.12–2	0.06–4	0.25–4	0.12–1	0.03–0.12	0.03–0.12	0.03–0.06	≤ 0.03	0.5–16
	MIC ₉₀	1	1	1	1	0.06	0.12	0.06	0.03	16
<i>Phoma</i> sp. (7)	GM	0.1	0.17	0.17	0.14	0.03	0.03	0.03	0.03	1.78
	Range	0.03–4	0.03–2	0.03–2	0.03–1	≤ 0.03	≤ 0.03	≤ 0.03	≤ 0.03	0.5–16
	MIC ₉₀	0.25	1	0.5	0.5	0.03	0.03	0.03	0.03	4
<i>Diaporthe sclerotoides</i> (4)	GM	0.06	0.21	2	0.5	0.04	0.03	0.03	0.03	4
	Range	0.03–0.12	0.12–0.25	1–4	0.5	0.03–0.06	≤ 0.03	≤ 0.03	≤ 0.03	0.5–16
	MIC ₉₀	0.12	0.25	2	0.5	0.03	0.03	0.03	0.03	8
<i>Pyrenochaetopsis leptospora</i> (4)	GM	0.7	0.59	0.7	0.21	0.03	0.03	0.03	0.03	4
	Range	0.03–4	0.25–2	0.06–16	0.03–1	≤ 0.03	≤ 0.03	≤ 0.03	≤ 0.03	0.5–16
	MIC ₉₀	2	1	1	0.5	0.03	0.03	0.03	0.03	16
Overall (85)	GM	0.33	0.61	1	0.46	0.06	0.06	0.06	0.04	2.9
	Range	0.03–8	0.03–16	0.03–16	0.03–16	0.03–8	0.03–4	0.03–8	0.03–2	0.25–32
	MIC ₉₀	2	4	16	16	0.25	0.5	2	0.03	16

^aGM, geometric mean; MIC₉₀, drug concentration that inhibited 90% of isolates.^bAMB, amphotericin B; VRC, voriconazole; ITC, itraconazole; PSC, posaconazole; AFG, anidulafungin; CFG, caspofungin; MFG, micafungin; TRB, terbinafine; 5FC, flucytosine.

published. In general, the coelomycetous fungi are involved in many kinds of mycoses, with superficial to deep infections, onychomycosis, cutaneous infections, keratitis, and endophthalmitis being relatively frequent. In general, the most commonly reported species clinically are *Colletotrichum* spp. (13–20), *Neoscytalidium dimidiatum* (21–25), and *Phoma* spp. (11, 26–35). Our study partly confirms the data from previous studies in which *Neoscytalidium dimidiatum* (approximately 15%), *Paraconiothyrium cyclothyrioides* (approximately 7%), and *Phoma herbarum* (approximately 6.5%) were the most common species, having been recovered mainly from superficial tissue and respiratory tract specimens. However, of these fungi, the only species that is relatively easy to identify by phenotypic criteria is *N. dimidiatum*, which is the best known coelomycetous fungus found clinically (22, 23, 36). The identification of the other fungi mentioned above generally requires the use of molecular tools due to the difficulty of achieving *in vitro* sporulation. Although *Paraconiothyrium cyclothyrioides* was relatively common in our studied samples, there are only two clinical reports that refer to this species. Both

cases are from immunocompromised patients; in one case *P. cyclothyrioides* caused skin lesions of the lower extremities, and in the second case it produced a systemic coinfection together with *Phaeoacremonium parasiticum* (37, 38). Even though *Phoma* sporulates easily, it is commonly misidentified as other related genera, such as *Ascochyta*, because the genera have similar morphologies, physiologies, and nucleotide sequences (39, 40). Boerema et al. carried out one of the most comprehensive revisions of the taxonomy of the genus *Phoma*. Using systematic criteria that predominated then, approximately 220 species were accepted, distributed into nine sections (41). In a recent multilocus study based on the sequence data of the 18S nrRNA (SSU) and LSU genes, other authors demonstrated that such classification was totally artificial (42). Currently, *Phoma sensu stricto* is included in the family *Didymellaceae*, and the other *Phoma*-like fungi belong to other phylogenetic families, i.e., *Cucurbitariaceae*, *Leptosphaeriaceae*, *Phaeosphaeriaceae*, etc. (39, 40, 42, 43).

It is of note that one of the frequently isolated species in our study, *Didymella heteroderae* (5.2% of isolation frequency), has never been mentioned as an etiologic agent of human infections even though our results reveal its ability to grow and to sporulate at 37°C, which is uncommon in that genus and suggests its potential pathogenicity.

An important clinical presentation of the coelomycetous fungi is eumycetoma, which is restricted to a specific group of pleosporalean species of fungi, namely, *Medicopsis romeroi* (formerly, *Pyrenochaeta romeroi*) (44–46), *Biatrispora mackinnonii* (formerly *Pyrenochaeta mackinnonii*) (46), and *Trematosphaeria grisea* (formerly, *Madurella grisea*) (47–49), among others. However, in the present study only nine of the isolates that were isolated from superficial and, less frequently, from deep tissues belonged to these genera. This might be explained by the fact that the habitat of these fungi is usually restricted to arid zones of East Africa and India and, occasionally, South America (46, 50, 51).

Despite several studies in recent years devoted to infections by coelomycetous fungi, little clinical data exist. The first well-documented review of human infections caused by these fungi was carried out by Punithalingham (11), who referenced a total of 12 species belonging primarily to the genera *Botryodiplodia*, *Dothiorella*, *Hendersonula*, *Phoma*, *Phyllosticta*, *Pseudochaetosphaeronema*, and *Pyrenochaeta*. In that work, a morphological description of these taxa and their clinical origin was provided, together with a dichotomous key for their identification. However, in our study, just under 12% of the total isolates identified belonged to such genera. In a recent study, Stchigel and Sutton (4) provided detailed information about the species of these fungi isolated from clinical samples, described useful tools for their isolation and identification, and gave general guidelines for infection management and treatment. These authors concluded that these organisms are easy to isolate but that it was difficult to induce *in vitro* fructification and sporulation. Our results are in agreement with theirs because 43% of our isolates failed to sporulate, and it was only possible to identify them and to determine their phylogenetic relationships by DNA sequencing.

The prevalence of coelomycetous fungi found in these clinical specimens—more than 200 isolates recovered in a 9-year period—goes against the fact that so few studies have described infections by them. This highlights the difficulty in conducting a comprehensive study of these fungi and in establishing their real occurrence in clinical settings. The taxonomy of these fungi is very complex because numerous isolates are usually unable to sporulate *in vitro* or to produce different synanamorphs, which sometimes predominate over the traditional coelomycete structures, making their phenotypic recognition difficult; reliable identification can be done, therefore, only by gene sequencing (9, 46, 52). However, even in this case, there are a very high number of genera and species of coelomycetous fungi, and the phylogenetic boundaries of numerous taxa are still unresolved. Therefore, we carried out a phylogenetic analysis of a large set of coelomycetous fungi using LSU sequences. This marker proved useful for solving the phylogeny of most of the isolates included in the study, identi-

fying them, at least at genus level, and showing, in front of the internal transcribed spacer (ITS), the advantage of an easy alignment of sequences.

The increasing use of molecular tools in fungal taxonomy has allowed the recognition of numerous new taxa that are impossible to detect by traditional methods. Recently, several new species of coelomycetous fungi, namely, *Rousoella percutanea*, *Truncatella angustata*, *Hongkongmyces pedis*, *Rhytidhysterion* spp., *Pseudochaetosphaeronema martinelli*, and *Emarellia* spp., have been involved in cases of subcutaneous infections and eumycetoma (53–58), and some of our *Pleosporales* isolates, having failed to sporulate, could represent new taxa.

Although clinical breakpoints for coelomycetous fungi have not been defined and although *in vitro* antifungal susceptibility studies on these fungi are scarce, most of the species seem to be inhibited by amphotericin B (4). Our results show that posaconazole is the most active of the triazoles tested, and results for amphotericin B are similar *in vitro* to those reported by Chowdhary et al. (10). Currently, only disseminated infections due to *N. dimidiatum* have been conducted in animal models, and amphotericin B, voriconazole, and posaconazole have been shown to be effective in the treatment of this experimental mycosis (36). Guidelines for the management of infections due to coelomycetous fungi include only a small group of taxa (*Neoscytalidium*, *Phoma*, and *Pyrenochaeta* spp.) (10) although our study supports those protocols. A recent study by Guégan et al. (59) analyzed several coelomycetous fungi that were implicated in human mycosis and concluded that the surgical resection of infected tissues is advisable for treating well-delimited lesions and that surgery together with new triazoles could be used if lesions are extensive.

In conclusion, this study demonstrates that a wide variety of fungal taxa, identified through their morphology as coelomycetous fungi, are involved in human infections in the United States. However, more studies are necessary to understand the real prevalence of coelomycete infections throughout the world. The most active antifungal drugs to treat them seem to be terbinafine, echinocandins, and amphotericin B, while results for the azoles varied. Although the LSU gene sequence is useful for preliminary identification and for establishing phylogenetic relationships between the majority of coelomycetous fungi, future molecular studies testing a higher number of genes are essential to properly identify doubtful isolates at the species level.

MATERIALS AND METHODS

Fungal isolates and sequences. A total of 230 isolates of coelomycetous fungi were included in this study, consisting of 224 from human clinical specimens, 3 from animal sources, and 3 from environmental samples. All of the isolates were provided by the Fungus Testing Laboratory of the University of Texas Health Science Center at San Antonio (UTHSC; San Antonio, Texas, USA). In addition, 92 D1-D2 sequences corresponding to type or reference strains were retrieved from GenBank and CBS databases and included in the phylogenetic analysis.

Morphological and physiological characterization. For cultural characterization, the isolates were grown on oatmeal agar (OA; 30 g of filtered oat flakes, 15 g of agar-agar, 1 liter of tap water) and malt extract agar (MEA; 40 g of malt extract, 15 g of agar-agar, 1 liter of distilled water) at $20 \pm 1^\circ\text{C}$ for 14 days in darkness. The ability of the isolates to grow at 37°C was determined on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) after 7 days of incubation in darkness. The morphological features of the vegetative and reproductive structures were studied using an Olympus CH2 light-field microscope (Olympus Corporation, Tokyo, Japan) in wet mounts (on water and lactic acid) and slide cultures (isolates grown on OA and MEA). The isolates were characterized phenotypically according to traditional criteria (4, 5, 41, 60). Color standards are from Kornerup and Wanscher (61). Photomicrographs were taken with an Axio-Imager M1 light-field microscope (Zeiss, Oberkochen, Germany).

DNA extraction, amplification, and sequencing. The total genomic DNA was extracted from colonies grown on PDA after 7 days of incubation at $20 \pm 1^\circ\text{C}$, using a FastDNA kit protocol (Bio101; Vista, CA) with a FastPrep FP120 instrument (Thermo Savant, Holbrook, NY) according to the manufacturer's protocol. DNA was quantified using a NanoDrop 2000 instrument (Thermo Scientific, Madrid, Spain). The D1-D2 domains were amplified with the primer pair LR0R and LR5 (62). The amplicons were sequenced in both directions with the same primer pair used for amplification at Macrogen Europe (Macrogen, Inc., Amsterdam, The Netherlands). The consensus sequences were obtained using SeqMan software, version 7.0.0 (DNASTar Lasergene, Madison, WI, USA).

Molecular identification and phylogenetic analysis. Preliminary molecular identification of the isolates was made using the D1-D2 nucleotide sequences in blastn searches (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the CBS database (www.cbs.knaw.nl). Only the sequences of type or reference strains

deposited in CBS/GenBank databases were considered for identification purposes. A level of identity of $\geq 98\%$ was considered for species-level identification.

For the phylogenetic study, the sequences were aligned using the ClustalW application (63) of the MEGA, version 6.06 (64), computer program, refined with MUSCLE (65), and manually adjusted using the same software platform. Phylogenetic reconstructions were made by maximum-likelihood (ML) and Bayesian inference (BI) with MEGA, version 6.06, and MrBayes, version 3.2.4 (66), respectively. The best substitution model for the gene matrix (general time-reversal model incorporating invariable sites and a discrete gamma distribution [GTR+I+G]) was estimated using MrModelTest, version 2.3 (67). For ML analyses, a nearest-neighbor interchange was used as the heuristic method for tree inference. Support for internal branches was assessed by 1,000 ML bootstrapped pseudoreplicates. Bootstrap support (BS) of ≥ 70 was considered significant. For BI analyses, Markov chain Monte Carlo (MCMC) sampling was carried out with 23 million generations, with samples taken every 1,000 generations. The 50% majority rule consensus trees and posterior probability values (PP) were calculated after the first 25% of the resulting trees was removed for burn-in. A PP value of ≥ 0.95 was considered significant. *Saccharomyces castellii* (NRRL Y-12630; GenBank accession number [AY048167](#)) and *Saccharomyces cerevisiae* (NRRL Y-12632; GenBank accession number [AY048154](#)) were used as outgroups.

Antifungal susceptibility testing. Using a broth microdilution reference method (68), the *in vitro* antifungal susceptibilities of 85 isolates were determined of selected species of the genera *Colletotrichum*, *Diaporthe*, *Didymella*, *Epicoccum*, *Neoscytalidium*, *Paraconiothyrium*, *Phoma* sp., and *Pyrenochaetopsis*. The following antifungals were tested: amphotericin B, voriconazole, posaconazole, itraconazole, caspofungin, anidulafungin, micafungin, terbinafine, and flucytosine. The minimal effective concentration (MEC) was determined after 48 h for the echinocandins, and the MIC was determined after 48 h and 72 h for the other drugs. *Candida parapsilosis* ATCC 22019 and *Paecilomyces variotii* ATCC MYA-3630 were used as controls. The inocula for the coelomycetous fungi that did not sporulate were prepared according to the method of Chowdhary et al. (69).

Accession number(s). The DNA sequences determined in this study have been deposited in GenBank under accession numbers [LN907285](#) to [LN907514](#).

ACKNOWLEDGMENTS

This work was supported by the Spanish Ministerio de Economía y Competitividad, grant CGL2013-43789-P.

We have no conflicts of interest to declare.

REFERENCES

- Kirk PM, Cannon PF, Stalpers JA, Minter DW. 2008. Ainsworth & Bisby's dictionary of the fungi, 10th ed. CAB International, Wallingford, United Kingdom.
- Sutton DA. 1999. Coelomycetous fungi in human disease. A review: clinical entities, pathogenesis, identification and therapy. *Rev Iberoam Micol* 16:171–179.
- Revankar SG, Sutton DA. 2010. Melanized fungi in human disease. *Clin Microbiol Rev* 23:884–928. <https://doi.org/10.1128/CMR.00019-10>.
- Stchigel AM, Sutton DA. 2013. Coelomycete fungi in the clinical lab. *Curr Fungal Infect Rep* 7:171–191. <https://doi.org/10.1007/s12281-013-0139-9>.
- Sutton BC. 1980. The Coelomycetes. Fungi Imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological, Kew, England.
- Nag Raj TR. 1993. Coelomycetous anamorphs with appendage-bearing conidia. Mycologue Publications, Waterloo, Ontario, Canada.
- Schoch CL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI, de Gruyter J, de Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y, Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, Hyde KD, Jones EBG, Kohlmeyer J, Kruys A, Li YM, Lücking R, Lumbsch HT, Marvanová L, Mbatchou JS, McVay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP, Nelson P, Owensby CA, Phillips AJL, Phongpaichit S, Pointing SB, Pujade-Renaud V, Raja HA, Plata ER, Robertse B, Ruibal C, Sakayaroj J, Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S, Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg JHC, Yonezawa H, Zhang Y, Spatafora JW. 2009. A class-wide phylogenetic assessment of *Dothideomycetes*. *Stud Mycol* 64:1–15. <https://doi.org/10.3114/sim.2009.64.01>.
- Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW. 2014. *Pestalotiopsis* revisited. *Stud Mycol* 79:121–186. <https://doi.org/10.1016/j.simyco.2014.09.005>.
- Wijayawardene NN, Hyde KD, Wanasinghe DN, Papizadeh M, Goonasekara ID, Camporesi E, Bhat DJ, McKenzie EHC, Phillips AJL, Diederich P, Tanaka K, Li WJ, Tangthirasunun N, Phookamsak R, Dai D-Q, Dissanayake AJ, Weerakoon G, Maharachchikumbura SSN, Hashimoto A, Matsumura M, Bahkali AH, Wang Y. 2016. Taxonomy and phylogeny of dematiaceous coelomycetes. *Fungal Divers* 77:1–316. <https://doi.org/10.1007/s13225-016-0360-2>.
- Chowdhary A, Meis JF, Guarro J, de Hoog GS, Kathuria S, Arendrup MC, Arikian-Akdagli S, Akova M, Boekhout T, Caira M, Guinea J, Chakrabarti A, Dannaoui E, van Diepeningen A, Freiburger T, Groll AH, Hope WW, Johnson E, Lackner M, Lagrou K, Lanternier F, Lass-Flörl C, Lortholary O, Meletiadis J, Muñoz P, Pagano L, Petrikos G, Richardson MD, Roilides E, Skiada A, Tortorano AM, Ullmann AJ, Verweij PE, Cornely OA, Cuenca-Estrella M. 2014. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of systemic phaeohyphomycosis: diseases caused by black fungi. *Clin Microbiol Infect* 20:47–75. <https://doi.org/10.1111/1469-0691.12515>.
- Punithalingam E. 1979. Sphaeropsidales in culture from humans. *Nova Hedwigia* 31:119–158.
- Liu JK, Phookamsak R, Dai DQ, Tanaka K, Jones EG, Xu JC, Chuksatitiro E, Hyde K. 2014. *Roussioellaceae*, a new pleosporalean family to accommodate the genera *Neoroussioella* gen. nov., *Roussioella* and *Roussioellopsis*. *Phytotaxa* 181:1–33. <https://doi.org/10.11646/phytotaxa.181.1.1>.
- Guarro J, Svidzinski TE, Zaror L, Forjaz MH, Gené J, Fischman O. 1998. Subcutaneous hyalohyphomycosis caused by *Colletotrichum gloeosporioides*. *J Clin Microbiol* 36:3060–3065.
- Cano J, Guarro J, Gené J. 2004. Molecular and morphological identification of *Colletotrichum* species of clinical interest. *J Clin Microbiol* 42: 2450–2454. <https://doi.org/10.1128/JCM.42.6.2450-2454.2004>.
- Castro LG, Da Silva Lacaz C, Guarro J, Gené J, Heins-Vaccari EM, de Freitas Leite RS, Arriagada GL, Reguera MM, Ito EM, Valente NY, Nunes RS. 2001. Phaeohyphomycotic cyst caused by *Colletotrichum crassipes*. *J Clin Microbiol* 39:2321–2324. <https://doi.org/10.1128/JCM.39.6.2321-2324.2001>.
- Fernandez V, Dursun D, Miller D, Alfonso EC. 2002. *Colletotrichum kera-titis*. *Am J Ophthalmol* 134:435–438. [https://doi.org/10.1016/S0002-9394\(02\)01576-3](https://doi.org/10.1016/S0002-9394(02)01576-3).
- Chakrabarti A, Shivaprakash MR, Singh R, Tarai B, George VK, Fomda BA, Gupta A. 2008. Fungal endophthalmitis: fourteen years' experience from a center in India. *Retina* 28:1400–1407. <https://doi.org/10.1097/IAE.0b013e318185e943>.

18. Shivaprakash MR, Appannanavar SB, Dhaliwal M, Gupta A, Gupta S, Gupta A, Chakrabarti A. 2011. *Colletotrichum truncatum*: An unusual pathogen causing mycotic keratitis and endophthalmitis. *J Clin Microbiol* 49:2894–2898. <https://doi.org/10.1128/JCM.00151-11>.
19. Shiraishi A, Araki-Sasaki K, Mitani A, Miyamoto H, Sunada A, Ueda A, Asari S, Zheng X, Yamamoto Y, Hara Y, Ohashi Y. 2011. Clinical characteristics of keratitis due to *Colletotrichum gloeosporioides*. *J Ocul Pharmacol Ther* 27:487–491. <https://doi.org/10.1089/jop.2011.0011>.
20. Figtree M, Weeks K, Chan L, Leyton A, Bowes A, Giuffre B, Sullivan M, Hudson BJ. 2013. *Colletotrichum gloeosporioides* sensu lato causing deep soft tissue mycosis following a penetrating injury. *Med Mycol Case Rep* 2:40–43. <https://doi.org/10.1016/j.mmcr.2013.01.003>.
21. al-Rajhi AA, Awad AH, Al-Hedaithy SS, Forster RK, Caldwell KC. 1993. *Scytalidium dimidiatum* fungal endophthalmitis. *Br J Ophthalmol* 77:388–390. <https://doi.org/10.1136/bjo.77.6.388>.
22. Elewski BE. 1996. Onychomycosis caused by *Scytalidium dimidiatum*. *J Am Acad Dermatol* 35:336–338. [https://doi.org/10.1016/S0190-9622\(96\)90664-7](https://doi.org/10.1016/S0190-9622(96)90664-7).
23. Madrid H, Ruiz-Cendoya M, Cano J, Stchigel A, Orofino R, Guarro J. 2009. Genotyping and in vitro antifungal susceptibility of *Neoscytalidium dimidiatum* isolates from different origins. *Int J Antimicrob Agents* 34:351–354. <https://doi.org/10.1016/j.ijantimicag.2009.05.006>.
24. Machouart M, Menir P, Helenon R, Quist D, Desbois N. 2013. *Scytalidium* and scytalidiosis: what's new in 2012? *J Mycol Med* 23:40–46. <https://doi.org/10.1016/j.mycmed.2013.01.002>.
25. Bakshizadeh M, Hashemian HR, Najafzadeh MJ, Dolatabadi S, Zarrinfar H. 2014. First report of rhinosinusitis caused by *Neoscytalidium dimidiatum* in Iran. *J Med Microbiol* 63:1017–1019. <https://doi.org/10.1099/jmm.0.065292-0>.
26. Bakerspigel A. 1970. The isolation of *Phoma hibernica* from lesions on a leg. *Sabouraudia* 7:261–264.
27. Punithalingam E. 1976. *Phoma oculo hominis* sp. nov. from corneal ulcer. *Trans Br Mycol Soc* 67:142–143. [https://doi.org/10.1016/S0007-1536\(76\)80022-8](https://doi.org/10.1016/S0007-1536(76)80022-8).
28. Bakerspigel A, Lowe D, Rostas A. 1981. The isolation of *Phoma eupyrena* from a human lesion. *Arch Dermatol* 117:362–363. <https://doi.org/10.1001/archderm.1981.01650060052024>.
29. Shukla NP, Rajak RK, Agarwal GP, Gupta D. 1984. *Phoma minutispora* as a human pathogen. *Mykosen* 27:255–258.
30. Baker JG, Salkin IF, Forgacs P, Haines JH, Kemna ME. 1987. First report of subcutaneous phaeohyphomycosis of the foot caused by *Phoma minutella*. *J Clin Microbiol* 25:2395–2397.
31. Rai MK. 1989. *Phoma sorghina* infection in human being. *Mycopathologia* 105:167–170. <https://doi.org/10.1007/BF00437250>.
32. Rosen T, Rinaldi MJ, Tschen JA, Stern JK, Cernoch P. 1996. Cutaneous lesions due to *Pleurophoma* (*Phoma*) complex. *South Med J* 89:431–434. <https://doi.org/10.1097/00007611-199604000-00018>.
33. Hirsh AH, Schiff TA. 1996. Subcutaneous phaeohyphomycosis caused by an unusual pathogen: *Phoma* species. *J Am Acad Dermatol* 34:679–680. [https://doi.org/10.1016/S0190-9622\(96\)80083-1](https://doi.org/10.1016/S0190-9622(96)80083-1).
34. Tullio V, Banche G, Allizond V, Roana J, Mandras N, Scalas D, Panzone M, Cervetti O, Valle S, Carloni N, Cuffini AM. 2010. Non-dermatophyte moulds as skin and nail foot mycosis agents: *Phoma herbarum*, *Chaetomium globosum* and *Microascus cinereus*. *Fungal Biol* 114:345–349. <https://doi.org/10.1016/j.funbio.2010.02.003>.
35. Roehm CE, Salazar JC, Hagstrom N, Valdez TA. 2012. *Phoma* and *Acremonium* invasive fungal rhinosinusitis in congenital acute lymphocytic leukemia and literature review. *Int J Pediatr Otorhinolaryngol* 76:1387–1391. <https://doi.org/10.1016/j.ijporl.2012.06.026>.
36. Ruiz-Cendoya M, Madrid H, Pastor J, Guarro J. 2010. Evaluation of antifungal therapy in a neutropenic murine model of *Neoscytalidium dimidiatum* infection. *Int J Antimicrob Agents* 35:152–155. <https://doi.org/10.1016/j.ijantimicag.2009.09.028>.
37. Gordon RA, Sutton DA, Thompson EH, Shrikanth V, Verkley GJ, Stielow JB, Mays R, Oleske D, Morrison LK, Lapolla WJ, Galfione S, Tyring S, Samathanam CA, Fu J, Wickes BL, Mulanovich V, Wanger A, Arias CA. 2012. Cutaneous phaeohyphomycosis caused by *Paraconiothyrium cyclothyrioides*. *J Clin Microbiol* 50:3795–3798. <https://doi.org/10.1128/JCM.01943-12>.
38. Colombier MA, Alanio A, Denis B, Melica G, Garcia-Hermoso D, Levy B, Peraldi MN, Glotz D, Bretagne S, Gallien S. 2015. Dual invasive infection with *Phaeoacremonium parasiticum* and *Paraconiothyrium cyclothyrioides* in a renal transplant recipient: Case report and comprehensive review of the literature of *Phaeoacremonium* phaeohyphomycosis. *J Clin Microbiol* 53:2084–2094. <https://doi.org/10.1128/JCM.00295-15>.
39. Aveskamp MM, de Gruyter J, Woudenberg JH, Verkley GJ, Crous PW. 2010. Highlights of the *Didymellaceae*: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. *Stud Mycol* 65:1–60. <https://doi.org/10.3114/sim.2010.65.01>.
40. Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW. 2015. Resolving the *Phoma* enigma. *Stud Mycol* 82:137–217. <https://doi.org/10.1016/j.simyco.2015.10.003>.
41. Boerema GH, de Gruyter J, Noordeloos ME, Hamers M. 2004. *Phoma* identification manual. Differentiation of specific and infraspecific taxa in culture. CABI Publishing, Cambridge, United Kingdom.
42. de Gruyter J, Aveskamp MM, Woudenberg JH, Verkley GJ, Groenewald JZ, Crous PW. 2009. Molecular phylogeny of *Phoma* and allied anamorph genera: towards a reclassification of the *Phoma* complex. *Mycol Res* 113:508–519. <https://doi.org/10.1016/j.mycres.2009.01.002>.
43. de Gruyter J, Woudenberg JH, Aveskamp MM, Verkley GJ, Groenewald JZ, Crous PW. 2013. Redisposition of *Phoma*-like anamorphs in *Pleosporales*. *Stud Mycol* 75:1–36. <https://doi.org/10.3114/sim0004>.
44. Borelli D. 1979. Opportunistic fungi as producers of gray colonies and mycetomata. *Dermatologica* 159:168–174. <https://doi.org/10.1159/000250685>.
45. Mathuram Thiagarajan U, Bagul A, Nicholson ML. 2011. A nodulo-cystic eumycetoma caused by *Pyrenochaeta romeroi* in a renal transplant recipient: a case report. *J Med Case Rep* 5:460. <https://doi.org/10.1186/1752-1947-5-460>.
46. Ahmed SA, Van De Sande WW, Stevens DA, Fahal A, Van Diepeningen AD, Menken SB, de Hoog GS. 2014. Revision of agents of black-grain eumycetoma in the order *Pleosporales*. *Persoonia* 33:141–154. <https://doi.org/10.3767/003158514X684744>.
47. Butz WC, Ajello L. 1971. Black grain mycetoma: a case due to *Madurella grisea*. *Arch Dermatol* 104:197–201. <https://doi.org/10.1001/archderm.1971.04000200085015>.
48. Gulati V, Bakare S, Tibrewal S, Ismail N, Sayani J, Baghla DPS. 2012. A rare presentation of concurrent *Scedosporium apiospermum* and *Madurella grisea* eumycetoma in an immunocompetent host. *Case Rep Pathol* 2012:154201. <https://doi.org/10.1155/2012/154201>.
49. de Hoog GS, Van Diepeningen AD, Mahgoub ES, Van de Sande WWJ. 2012. New species of *Madurella*, causative agents of black-grain mycetoma. *J Clin Microbiol* 50:988–994. <https://doi.org/10.1128/JCM.05477-11>.
50. Mackinnon JE, Ferrada-Urzuá LV, Montemayor L. 1943. *Madurella grisea* n. sp. *Mycopathologia* 4:384–393. <https://doi.org/10.1007/BF01237166>.
51. McGinnis MR. 1996. Mycetoma. *Dermatol Clin* 41:97–104.
52. Borman AM, Desnos-Ollivier M, Campbell CK, Bridge PD, Dannaoui E, Johnson EM. 2016. Novel Taxa Associated with Human Fungal Black-Grain Mycetomas: *Emarellia grisea* gen. nov., sp. nov., and *Emarellia paragrisea* sp. nov. *J Clin Microbiol* 54:1738–1745. <https://doi.org/10.1128/JCM.00477-16>.
53. Ahmed SA, Stevens DA, Van de Sande WWJ, Meis JF, de Hoog GS. 2014. *Roussioella percutanea*, a novel opportunistic pathogen causing subcutaneous mycoses. *Med Mycol* 52:689–698. <https://doi.org/10.1093/mmy/myu035>.
54. Jagielski T, Zak I, Tyrak J, Bryk A. 2015. First probable case of subcutaneous infection due to *Truncatella angustata*: a new fungal pathogen of humans? *J Clin Microbiol* 53:1961–1964. <https://doi.org/10.1128/JCM.00400-15>.
55. Tsang CC, Chan JF, Trendell-Smith NJ, Ngan AH, Ling IW, Lau SK, Woo PC. 2014. Subcutaneous phaeohyphomycosis in a patient with IgG4-related sclerosing disease caused by a novel ascomycete, *Hongkongmyces pedis* gen. et sp. nov.: first report of human infection associated with the family *Lindgomycetaceae*. *Med Mycol* 52:736–747. <https://doi.org/10.1093/mmy/myu043>.
56. Mahajan VK, Sharma V, Prabha N, Thakur K, Sharma NL, Rudramurthy SM, Chauhan PS, Mehta KS, Abhinav C. 2014. A rare case of subcutaneous phaeohyphomycosis caused by a *Rhytidhysterion* species: a clinico-therapeutic experience. *Int J Dermatol* 53:1485–1489. <https://doi.org/10.1111/ijd.12529>.
57. Mishra K, Das S, Goyal S, Gupta C, Rai G, Ansari MA, Saha R, Singal A. 2014. Subcutaneous mycoses caused by *Rhytidhysterion* species in an immunocompetent patient. *Med Mycol Case Rep* 5:32–34. <https://doi.org/10.1016/j.mmcr.2014.07.002>.
58. Ahmed SA, Desbois N, Quist D, Miossec C, Atoche C, Bonifaz A, de Hoog GS. 2015. Phaeohyphomycosis caused by a novel species, *Pseudochaeto-*

- sphaeronema martinelli*. J Clin Microbiol 53:2927–2934. <https://doi.org/10.1128/JCM.01456-15>.
59. Guégan S, García-Hermoso D, Sitbon K, Ahmed S, Moguelet P, Dromer F, Lortholary O, French Mycosis Study Group. 2016. Ten-year experience of cutaneous and/or subcutaneous infections due to coelomycetes in France. Open Forum Infect Dis 3:ofw106. <https://doi.org/10.1093/ofid/ofw106>.
60. de Hoog GS, Guarro J, Gené J, Figueras MJ. 2000. Atlas of clinical fungi, 2nd ed. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
61. Kornerup A, Wanscher JH. 1978. Methuen handbook of colour, 3rd ed. Methuen, London, England.
62. Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>.
63. Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680. <https://doi.org/10.1093/nar/22.22.4673>.
64. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30:2725–2729. <https://doi.org/10.1093/molbev/mst197>.
65. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. <https://doi.org/10.1093/nar/gkh340>.
66. Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>.
67. Nylander JA. 2004. MrModeltest v2. Uppsala University, Uppsala, Sweden.
68. Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, 2nd ed. Document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
69. Chowdhary A, Kathuria S, Singh PK, Agarwal K, Gaur SN, Roy P, Randhawa HS, Meis JF. 2013. Molecular characterization and in vitro antifungal susceptibility profile of *Schizophyllum commune*, an emerging basidiomycete in bronchopulmonary mycoses. Antimicrob Agents Chemother 57:2845–2848. <https://doi.org/10.1128/AAC.02619-12>.